Impact of High Nighttime Temperature on Respiration, Membrane Stability, Antioxidant Capacity, and Yield of Rice Plants

Abdul-Razack Mohammed and Lee Tarpley*

ABSTRACT

Nighttime temperature is one of the major environmental factors influencing plant metabolic processes. The respiration rates, membrane thermal stability (MTS), and total antioxidant capacities of leaves were investigated in rice (*Oryza sativa* L.) plants when exposed to high nighttime temperature (HNT) (32°C) or ambient nighttime temperature (ANT) (27°C), and with or without potential preventive exogenous effector (chemical) treatments. The exogenous effector treatments included α-tocopherol (vitamin E), glycine betaine, and salicylic acid, which play important but different roles in inducing thermal tolerance in many plant species. Plants were subjected to an HNT through use of continuously controlled infrared heaters, starting from 2000 h until 0600 h. High nighttime temperature increased respiration rates, decreased MTS, and negatively affected the yield (by 95%). Application of salicylic acid somewhat lowered the reduction in yield due to HNT (76 vs. 95%) by decreasing the respiration rates and increasing MTS and total antioxidant capacity of rice plants.

Abdul-Razack Mohammed and Lee Tarpley, Texas AgriLife Research and Extension Center, 1509 Aggie Drive, Beaumont, TX 77713. Received 19 Mar. 2008. *Corresponding author (ltarpley@tamu.edu).

Abbreviations: ANT, ambient nighttime temperature; DAE, days after emergence; EFG, early grain fill; GB, glycine betaine; HNT, high nighttime termperature; PPFD, photosynthetic photon flux density; ROS, reactive oxygen species; MD, mid-dough; MTS, membrane thermal stability.

Rice (*Oryza sativa* L.), which is the stable food of over half the world's population (Khush, 1997), is cultivated under a wide range of environments between latitudes 45°N and 40°S (Grist, 1986). Global circulation models project that the global temperature is likely to increase by 1.4 to 5.8°C because of projected increase in the concentrations of all greenhouse gases by the end of the 21st century (IPCC, 2001; Section 9.3.3, p. 555). Nighttime temperatures are projected to increase more than daytime temperatures (Alward et al., 1999). Peng et al. (2004) reported an increase in nighttime temperature (increase by 1.13°C) over a period of 25 yr (1979 to 2003) at the International Rice Research Institute, Manila, Philippines. A rise in nighttime temperature by 1°C can reduce rice grain yield by 10% (Peng et al., 2004). Thus, identifying and developing management practices, such as the application of agrochemicals to prevent or negate the negative effects of high nighttime temperatures, can be beneficial for worldwide rice production and food stability.

A projected increase in plant respiration in response to climate warming is of serious concern, as respiratory processes could consume a larger portion of total photosynthates (Paembonan et al.,

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1992). Respiration is typically partitioned into the functional components of construction (growth) and of maintenance and ion uptake to facilitate our understanding of the impact of the environment on respiratory processes (Lambers, 1985; Amthor, 1986). Maintenance respiration is mainly associated with turnover of proteins and lipids and maintenance of ion concentration gradients across membranes (Penning de Vries, 1975) and is the most responsive to environmental changes (Ryan, 1991). High nighttime temperatures (HNT) are considered to be disadvantageous because they can stimulate respiration, thereby affecting yield (Zheng et al., 2002). Increased respiration rates as a result of high temperatures can lead to production of reactive oxygen species (ROS) in many plants (McDonald and Vanlerberghe, 2005). Under normal physiological conditions, the toxic effects of ROS are minimized by enzymic and nonenzymic antioxidants; however, under stress conditions oxidant levels can overwhelm the antioxidant levels, leading to cell damage (Kreiner et al., 2002). Increased cell damage as a result of ROS can decrease membrane thermal stability (MTS), thereby disrupting water, ion, and organic-solute movement across plant membranes, thus affecting carbon production, consumption, transport, and accumulation (Christiansen, 1978). A common method of evaluating damage to membranes is by examining MTS, which measures electrolyte leakage from tissues, such as leaves, subjected to stresses such as drought (Blum and Ebercon, 1981), heat (Sullivan, 1972), and freezing (Dexter, 1956). Membrane thermal stability was positively associated with yield performance in wheat (*Triticum esculentum* L.) under heat-stressed conditions (Reynolds et al., 1994).

The antioxidant concentration of a plant is closely associated with its stress tolerance in some circumstances (Smirnoff, 1995). The severity of ROS-induced damage depends on the antioxidant status of the plant. Plants pretreated with α-tocopherol (vitamin E), glycine betaine (GB), or salicylic acid (SA), the potential preventive exogenous effectors (chemicals) used in this study, showed induced thermal tolerance and protection against oxidative damage (Fryer, 1992; Diaz-Zorita et al., 2001; Larkindale and Knight, 2002). The α-tocopherol is one of the most effective single-oxygen quenchers and is a strong antioxidant, whereas GB enhances tolerance to high temperatures by protecting certain enzymes (e.g., RUBISCO and citrate synthase) against heat-induced inactivation (Caldas et al., 1999; Mäkelä et al., 2000). Salicylic acid plays an essential role in preventing oxidative damage in plants by detoxifying superoxide radicals (Bowler et al., 1992) and stabilizing trimers of heat shock transcription factors (Larkindale and Knight, 2002). Salicylic acid is also involved in calcium signaling (Kawano et al., 1998) and induces thermal tolerance (Larkindale and Knight, 2002). Despite the importance of antioxidants in stress tolerance, little is known about the response of rice thermal tolerance to these preventative exogenous effectors (vitamin E, GB, and SA).

Our principal objectives in the present study were to determine (i) the effects of high nighttime temperature on respiration, membrane stability, antioxidant capacity, and yield of rice plants; and (ii) whether application of the exogenous effectors (α-tocopherol, GB, and SA) can negate the negative effects of high nighttime temperatures on rice plants.

MATERIALS AND METHODS

Plant Culture

Three experiments were conducted in the greenhouse at the Texas AgriLife Research and Extension Center at Beaumont, Texas. Cocodrie, a common U.S. rice cultivar, was used in all three experiments. In the first experiment (Exp-I), a set of 12 plants, 3 plants per chemical (exogenous effector) treatment, was grown under ambient nighttime temperature (ANT), and another set was grown under HNT. In the second (Exp-II) and third (Exp-III) experiments, a set of 20 plants, 5 plants per chemical treatment, were grown under ANT, and another set was grown under HNT. Plants were grown in 3-L pots that were placed in a square box (0.84 m^2), 10 pots per box. The boxes were lined with black plastic (thickness = 0.15 mm; FILM-GARD, Minneapolis, MN) that served as a water reservoir. Pots were filled with a clay soil (fine montmorillonite and thermic Entic Pelludert [Chen et al., 1989]) that is common to rice farms in the area. At 20 d after emergence (DAE), the boxes were filled with water to approximately 3 cm above the top of the soil in each pot. A foam cover was placed over the water surface to prevent direct infrared heating of water. A three-way split application of nitrogen was used as described by Mohammed et al. (2007). Nitrogen was applied in the form of urea and ammonium sulfate, and phosphorus in the form of P_20_5. At planting, urea-N was applied at the rate of 113.5 kg ha^{-1} along with 45.4 kg ha^{-1} P_20_5. The second and third nitrogen fertilizations (both 79.5 kg ha^{-1} nitrogen in the form of ammonium sulfate) were applied 20 DAE and at the panicle-differentiation stage. The plants were well matched in terms of developmental stage, as indicated by tiller development, at the beginning of the heat treatments described below.

Temperature Treatments

There are three experiments presented in the study. The assignment of heat treatment to greenhouse location was random within each experiment. The average nighttime temperatures (between 2000 h to 0600 h) at Beaumont, Texas (30°4′0″ N, 94°16′59″ W), during the reproductive growth of the rice plants often ranged above 25°C (up to 28°C) (Fig. 1a). Hence, ANT was set at 27°C. The greenhouse was maintained at 27°C nighttime temperature and within this, plants of the HNT treatment were subjected to elevated nighttime temperature through the use of nearly continuously controlled (subsecond response) infrared heaters (Chromalox, Ogden, UT), which were positioned 1.0 m above the topmost part of the plants and provided infrared temperature enrichment. In ANT treatments, dummy heaters were provided to account for shading.

The single fixed-element radiant process heater is mounted in an aluminum housing (frame). The length and breadth of the frame is 77.8 cm and 9.4 cm, respectively. The length of the heating element is 57.8 cm, and the diameter is 1 cm. The working voltage of the heating element is 120 V. The operating wavelengths of the infrared heaters are well above 1200 nm, and the infrared heater output is negligible below 1200 nm (Omega, 2008). Hence, there was no significant emission of photo-morphogenic wavelengths. The infrared heaters were controlled using power semiconductor controllers (SCR Power Control, Watlow Electric Manufacturing Company, St. Louis, MO) to enable proportioning heating action instead of on–off action. Air temperatures surrounding the plants were controlled at predetermined set points (27°C and 32°C). When the temperature was below the set point as determined by the readings from the air-temperature thermocouples positioned within a few centimeters of the uppermost or reproductive parts of the plant, the power controller sends a signal to the infrared heaters, which provided short, slightly elevated heating events, as needed, to raise the temperature to the desired set point. Air temperatures were monitored and maintained within the set points ±0.5°C (Fig. 1b, c). The nighttime temperature was imposed from 2000 h until 0600 h starting at 20 DAE until harvest. Daytime temperature, nighttime temperature, and humidity were monitored using stand-alone sensor–loggers (HOBOs, Onset Computer Corporation, Bourne, MA) in both portions of the study (Fig. 1b, c).

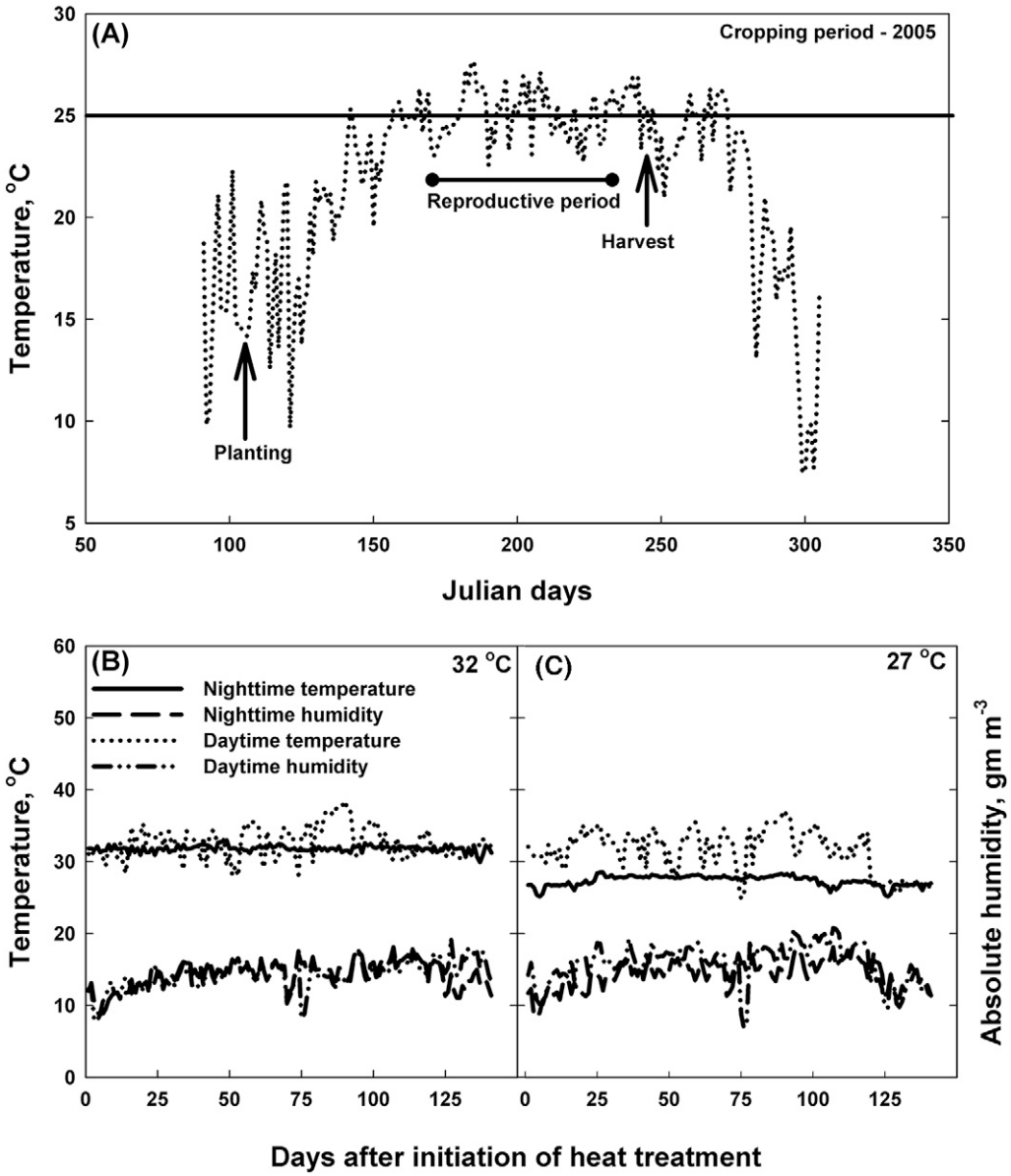

Figure 1. Average nighttime temperatures at Beaumont Research Center during (A) the cropping period and (B, C) average nighttime temperatures imposed during the course of the experiment using infrared heaters as well as average daytime temperature during the same period. (B, C) Average daytime and nighttime humidity are also shown under two nighttime temperature regimes.

Chemical Treatments

The α-tocopherol, GB, and SA were applied at the rate of 100 μL per application to the leaves using a precalibrated perfume-bottle sprayer. Each plant was treated three times (on a single day) to enable thorough coverage before imposing heat stress. The α-tocopherol was applied at the rate of 58 μmol per plant per spray (i.e., 580 mM); GB was applied at the rate of 182.3 μmole per plant per spray (i.e., 1.823 M), and SA, at the rate of 0.1 μmol per plant per spray (i.e., 1 mM). All the above chemicals were purchased from Sigma-Aldrich (St. Louis, MO), except for GB, which was supplied by Capstone Food Ingredients (Marion, MA).

Respiration Measurements

Leaf-level respiration was measured using an LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE). Respiration was measured on the penultimate leaf during the grain-filling stages. The area of the chamber was set to 6 cm^2, and the middle portion of the leaf was preferred for use while measuring respiration rates. While measuring respiration rates, the photosynthetic photon flux density (PPFD), provided by a 6400-02 LED light source, was set at 0 μmol m^{-2} s^{-1} (dark environment). The temperature and CO_2 concentration in the leaf cuvette were set at 25°C and 360 μmol, respectively. Respiration was measured during the nighttime between 2300 h and 0001 h at boot stage, early grain-fill (EGF) stage, and mid-dough (MD) stage.

Membrane Thermal Stability Assay

Leaves were harvested at EGF stage for the membrane thermal stability (MTS) assay. The MTS of leaves was measured using the procedure described by Martineau et al. (1979). Each sample assay consisted of two sets of five leaf discs cut with a 1-cm diameter punch from the penultimate leaves. The sets were placed into two separate test tubes with 10 mL of deionized water, after rinsing them three or four times with deionized water. One set of test tubes was submerged in a water bath at 55°C for 20 min to a depth equal to the height of water in the tubes. The other set of test tubes was held at room temperature (25°C). After that, both sets of test tubes were incubated at 10°C for 12 h. Conductance was measured using a conductivity meter (SensION5 Conductivity Meter, Hach Company, Loveland, CO) after standardizing with standard KCl solutions. Test tubes were then autoclaved for 20 min at 120°C at 0.15 Mpa and conductance was measured again as an indication of maximum potential leakage from a given sample (Ibrahim and Quick, 2001). The relative injury (RI) was calculated using the equation $RI = \{1-[1-(C_{55,i}/C_{55,f})]/[1-(C_{25,i}/C_{25,f})]\} \times 100$ where C_{55} and C_{25} refer to the conductance at 55°C and 25°C, respectively, and the subscripts i and f refer to the initial and final conductance.

Determination of Total Antioxidant Capacity

Total antioxidant capacity of the rice leaf was measured using the procedures (DPPH [2,2-diphenyl-1-picrylhydrazyl] assay) from Goffman and Bergman (2004), with modifications. For the DPPH assay, five leaf discs (0.0785 cm^2 each) were obtained from mid-blade while avoiding the mid-vein, and their weights recorded. Leaf discs were placed in a test tube (4-mL) with 1.5 mL methyl alcohol (MeOH) and then sealed. The test tubes were incubated at room temperature for 24 h in darkness to allow for complete extraction of antioxidants into the solution. Rice leaf extract of 40 μL was added to 960 μL DPPH (0.2 mM) solution and the optical density determined at 515 nm after 4 h. The antiradical efficiency of the rice leaf methanolic extracts was determined after 4 h by monitoring the reduction in the absorbance (515 nm) of the methanolic solution of DPPH with the extract. The values of DPPH after adding the extract was compared with those obtained from a blank solution of DPPH (zero antiradical activity). Trolox (6-Hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid) and DPPH standard curves were developed and the values were expressed in μM trolox equivalents g^{-1} leaf (fresh weight basis) using these standard curves. The DPPH and trolox were purchased from Sigma-Aldrich (St. Louis, MO). Total antioxidant capacity was measured at boot stage and MD stage of the rice plant.

Final harvest was performed between 110 and 114 DAE in experiments I, II, and III. For yield determination, panicles were harvested separately from other plant components and then dried at 70°C to constant weight. The panicles were threshed (HEGE 16 230 V, HEGE Maschinen, Domäne Hohebuch, D-74638 Waldenburg, Germany) and the collected paddy rice weighed.

Data Analysis

The experiments were laid out in a complete randomized design and were repeated for a total of three times. One set of plants was grown under ANT, whereas the other set was grown under HNT. In total, there were 52 plants in each set, 13 plants per chemical treatment. To test the significance of nighttime temperatures, the chemical treatments, and their interactive effects on respiration rates, electrolytic leakage, and total antioxidant capacities, the data were statistically analyzed using analysis of variance (ANOVA; SAS statistical analysis package version 9.0, SAS Institute, Cary, NC). The means were separated using Tukey's Least Significant Difference at an alpha level of 0.05. In addition, Hotellings T-square analysis, a special case of multivariate analysis of variance (MANOVA), was also performed on the data collected. Differences among the experiments, nighttime temperatures, chemicals, and their interactive effects were also tested. If there was no significant difference among the experiments for a parameter, then the values from all the experiments for that parameter were used to obtain the mean and error. The standard errors of the mean were also calculated and presented in the graphs as error bars.

RESULTS

The ANT and HNT were maintained as desired throughout the course of the experiments, and the humidity was similar between treatments (Fig. 1b, c). Mean daytime (0600 to 1900 h) and nighttime (1900 to 0600 h) temperatures during the cropping season (emergence to harvest) were 31.6 and 27.3°C, respectively, in ambient and 32.8 and 31.8°C, respectively, in ambient + 5°C temperature treatments. The daytime and nighttime humidity during the cropping season was 15.9 and 14.4 gm m^{-3}, respectively, in ambient and 14.3 and 14.4 gm m^{-3}, respectively, in ambient + 5°C temperature treatments (Fig. 1b, c).

There were no differences among the experiments for leaf respiration rates at boot or MD stage. However, at EGF stage in all three experiments, plants grown under both heat treatments (HNT and ANT) had 26 and 172% higher leaf respiration rates at EGF stage compared with boot and MD stages of the rice plants, respectively (Fig. 2). Leaf respiration was greater at 32°C compared with 27°C at boot stage, EGF stage, and MD stage (Table 1; Fig. 2). In all three experiments, leaf respiration rate, expressed as loss of carbon, was greater (27%) in plants grown under HNT compared with plants grown under ANT at EGF stage.

There were differences among the exogenous effector treatments (vitamin E, GB, and SA) for effect on leaf respiration rates at boot and EGF stages of rice plants in both the heat treatments. However, plants grown under HNT as well as ANT showed no differences among the chemical treatments for leaf respiration rates at MD stage (Table 1). At boot stage, plants treated with GB grown under HNT had greater loss (24%) of carbon compared with untreated plants in Exp-II and Exp-III. However, at EGF stage, on average, untreated plants grown under HNT showed greater loss of carbon (39%) compared with chemical-treated plants (Fig. 2). Plants grown under ANT responded differently to the chemical application than plants grown under HNT with

respect to leaf respiration rates. At boot stage, plants grown under ANT treated with SA showed 52 and 59% decrease in respiration rate compared with untreated plants in Exp-I and Exp-III, respectively. However, in Exp-II there were no differences between untreated plants and SA-treated plants. At EGF stage, untreated plants showed 25 and 35% increase in loss of carbon compared with the chemical-treated plants in Exp-I and Exp-II, respectively. However, in Exp-III, plants grown under ANT treated with vitamin E showed a greater loss (7%) in carbon compared with untreated plants at EGF stage (Fig. 2).

Electrolytic leakage expressed as relative injury measured at EGF stage was greater in plants grown under HNT compared with the plants grown under ANT (Fig. 3a). On average, plants grown under HNT showed a 60% increase in electrolytic leakage compared with plants grown under ANT. Application of vitamin E, GB and SA decreased electrolytic leakage by 31, 42, and 30%, respectively, compared with untreated plants grown under ANT (Fig. 3a). However, under HNT, control plants showed levels of leakage similar to other treatments except SA, where plants showed a 10% decrease in electrolytic leakage compared with untreated plants (Fig. 3a). There were no differences among the experiments for electrolytic leakage (Table 1).

Among the experiments, total antioxidant capacities measured at boot or MD stage of the rice plants were similar (Table 1). In addition, there were no differences between the heat treatments or among the chemical treatments with respect to total leaf antioxidant capacity. However, there was an interaction between the heat treatments and chemical treatments with respect to total leaf antioxidant capacity (Table 1). Plants grown under ANT showed no differences among the chemicals with respect to their effect on total leaf antioxidant capacity at boot stage as well as MD stage, except for plants treated with GB at boot stage (Fig. 4). Plants treated with GB showed a 30% increase in antioxidant capacity compared with untreated plants at boot stage. Plants grown under HNT and treated with SA showed increases of 30 and 16.7% in total antioxidant capacity at boot and MD stages, respectively (Fig. 4). The total antioxidant capacity declined as the plants treated with SA under HNT matured. However, there was an increase in total antioxidant capacity as the plants treated with GB under HNT matured. At MD stage, plants treated with GB grown under HNT showed a 17% increase in total antioxidant capacity (Fig. 4).

On average, plants grown under HNT showed a 90% decrease in yield compared with plants grown under ANT (Fig. 3b). Plants grown under ANT and treated with vitamin E, GB, and SA showed a 5.8, 12.7 and 13.5% increase in yield, respectively, compared with untreated plants. Similar results with respect to yield were seen in plants grown under HNT and treated with vitamin E, GB, and SA. Plants treated with vitamin E, GB, and SA showed a 4.5, 77, and 380% increase in yield, respectively, compared with untreated plants (Fig. 3b). The relative increases in yield as a result of GB and SA applications were different from untreated plants in both the heat treatments (Fig. 3b), whereas plants treated with vitamin E showed no difference from the untreated plants in either of the heat treatments. In addition, rice yields were similar across experiments (Table 1). Our results showed strong association between leaf respiration rates, electrolytic leakage, antioxidant capacities, and yield. Under HNT, an increase in antioxidant capacity was associated with a decrease in electrolytic leakage, which in turn was associated with a decrease in respiration rates (Fig. 4, Fig. 3a, and Fig. 2).

To evaluate patterns across the three parameters (respiration, electrolytic leakage, and antioxidant capacity), the values for these three parameters from the untreated (no exogenous effector) chemical treatment of the two heat treatments were plotted against each (Fig. 5). The values for these three parameters were also analyzed using Hotellings T-square analysis, which is a special case of MANOVA, for comparing two groups. The HN and ANT differed with significant variance explained by respiration and electrolytic leakage. High nighttime temperature increased respiration and electrolytic leakage of rice plants as compared with ANT (Fig. 5). However, HNT had no effect on total antioxidant capacity of rice plants. These three parameters—respiration, electrolytic leakage, and total antioxidant capacity—were strongly associated with yield. Hence, all three parameters are potentially influential on yield.

Table 1. Effects of experiment (Exp.), nighttime temperature (NT), and chemical (exogenous effector) treatment (Chem.) on respiration rates (RR), membrane thermal stability (MTS), total antioxidant capacity (TAC), and yield.

	RR Boot	RR EGF†	RR MD‡	MTS EGF	TAC Boot	TAC MD	Yield
Exp.	NS§	*	NS	NS	NS	NS	NS
NT	*	**	**	**	NS	NS	***
Chem.	*	***	NS	*	NS	NS	***
Exp. × NT	NS	NS	NS	NS	NS	NS	NS
Exp. × Chem	NS	NS	NS	NS	NS	NS	NS
NT × Chem.	NS	NS	NS	NS	*	*	NS
Exp × NT × Chem.	NS	NS	NS	NS	NS	NS	NS

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

†Early grain-fill (EGF) stage of rice plants.

‡Mid-dough (MD) stage of rice plants.

§NS, not significant.

DISCUSSION

Year-to-year variation in rice grain yield was attributed to changes in nighttime temperatures as a result of possible global warming (Peng et al., 2004). Our results indicated

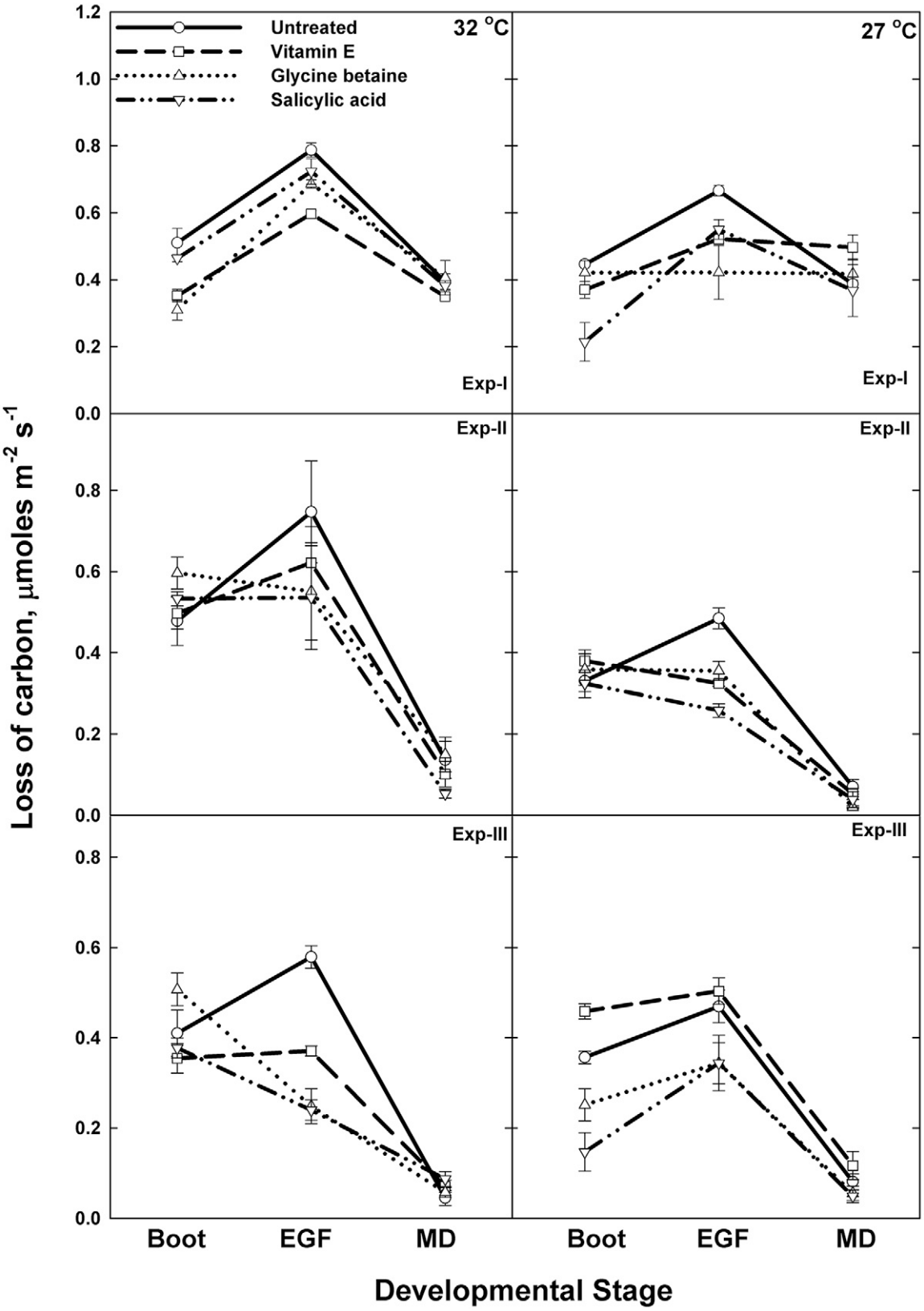

Figure 2. Temporal trends in leaf dark respiration rates of rice plants as affected by high nighttime temperature and exogenous effector (chemical) treatments. Leaf dark respiration rates were measured at boot, early grain-fill (EGF), and mid-dough (MD) stages. The error bars are shown if they are larger than the symbol, and represent SE. In Exp-I, the values are the average of three replications, whereas in Exp-II and III, the values are the average of five replications.

an increase in respiration rates in rice plants as a result of an increase in nighttime temperature, which was associated with a decrease in yield. Higher dark respiration increases the proportion of assimilates respired for maintenance and uncoupled respiration (Beevers, 1970), thereby affecting plant carbon status (Turnbull et al., 2002). Our results also indicated maximum respiration rates during EGF with decline as the plants matured (MD stage). Similar trends were seen in sunflower (*Helianthus annus* L.), wheat (*Triticum durum* L.), sorghum (*Sorghum bicolor* L. Moench.), and chickpea (*Cicer arietinum* L.), where the respiration rates peaked at grain-filling stage (Albrizio and Steduto, 2003) and then declined. The decline in the rates of leaf dark respiration toward the end of the crop cycle is associated with leaf senescence (Albrizio and Steduto, 2003).

Membrane thermal stability, when measured as the conductivity of electrolytes leaking from leaf disks at high temperature, has been suggested as one of the best techniques to evaluate the performance of a plant under high temperatures (Sullivan, 1972). Our results indicated decreased membrane stability in plants grown under HNT, indicating that high temperatures of the range used in the present study lead to leaky membranes. Previous studies reported increased electrolytic leakage as a result of increased temperature in cowpea (*Vigna unguiculata* L.) (Ismail and Hall, 1999; Ibrahim and Quick, 2001). The properties of the photosynthetic system, including key enzymes and thylakoid membrane activities depend on the thermal stability of membranes (Björkman et al., 1980). Moreover, it is well known that a functional cell-membrane system is central to crop yield productivity and adaptation of plants to high temperature (Raison et al., 1980). Hence, leaky membranes as a result of HNT can negatively affect crop productivity. In the present study, reduction in rice yields as a result of HNT was attributed to higher respiration rates and decreased MTS, in accordance with previous studies that reported decreased rice yields as a result of HNT (Ziska et al., 1996; Baker, 2004; Peng et al., 2004; Counce et al., 2005) and many studies that have reported decreased yields due to high temperatures as a result of increased leaf respiration rates (Lambers, 1985; Albrizio and Steduto, 2003) and leaf electrolytic leakage (Reynolds et al., 1994; Ismail and Hall, 1999).

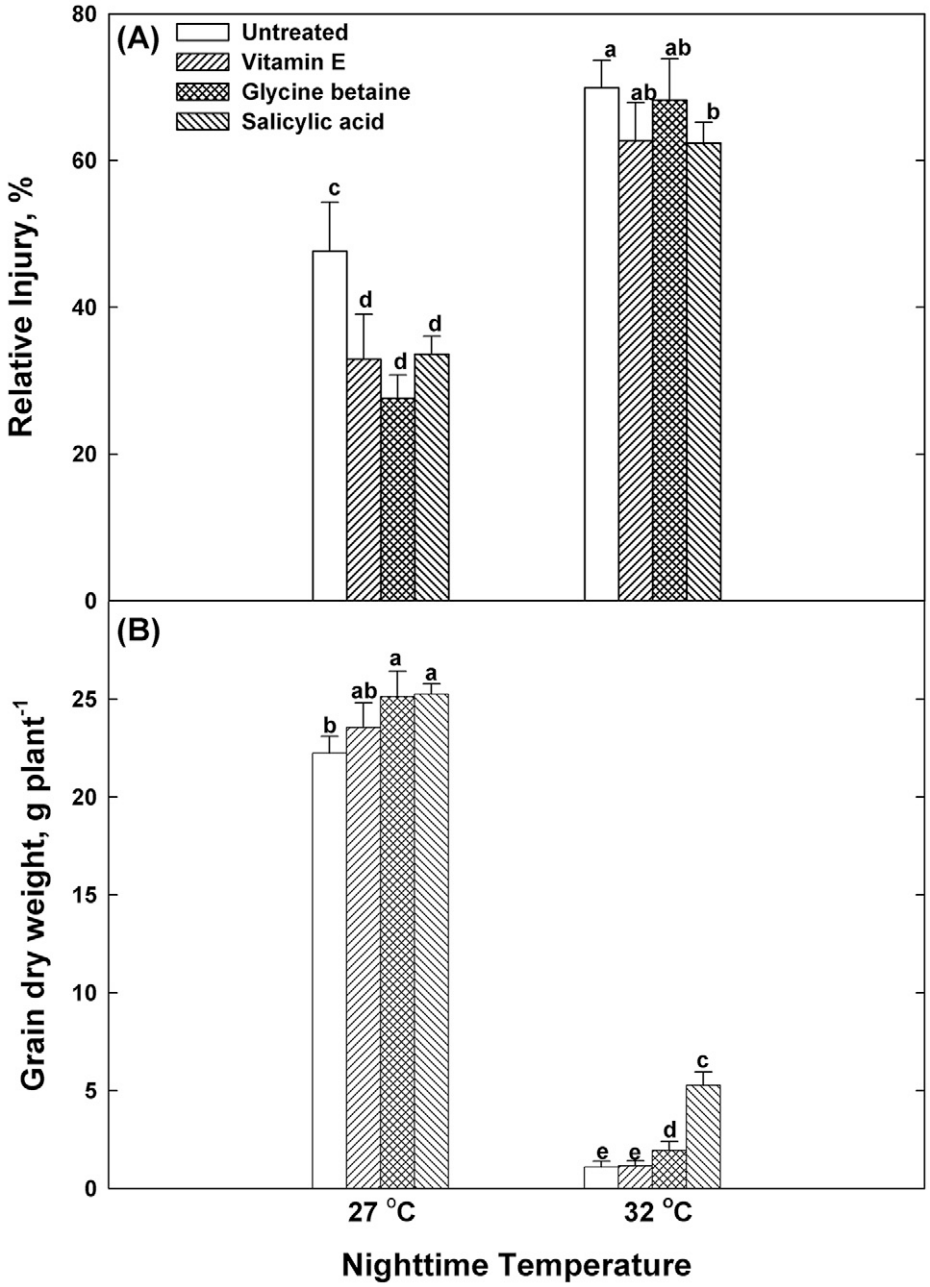

Figure 3. Effects of high nighttime temperature and exogenous effector (chemical) treatments on membrane thermal stability expressed as (A) relative injury and (B) grain dry weight. Membrane thermal stability was measured at early grain fill of the rice plant. The error bars are shown if they are larger than the symbol, and represent SE. The values are averages of the results of three independent experiments. In total, there were thirteen replications for each treatment.

Interpretation of the relationship between temperature, respiration, and yield is a difficult task, as it involves many other plant processes (Johnson and Thornley, 1985; Hemming et al., 2000). Previous studies have shown association of yield with respiration rates (Lambers, 1985), sugar and starch content (Turnbull et al., 2002), membrane stability

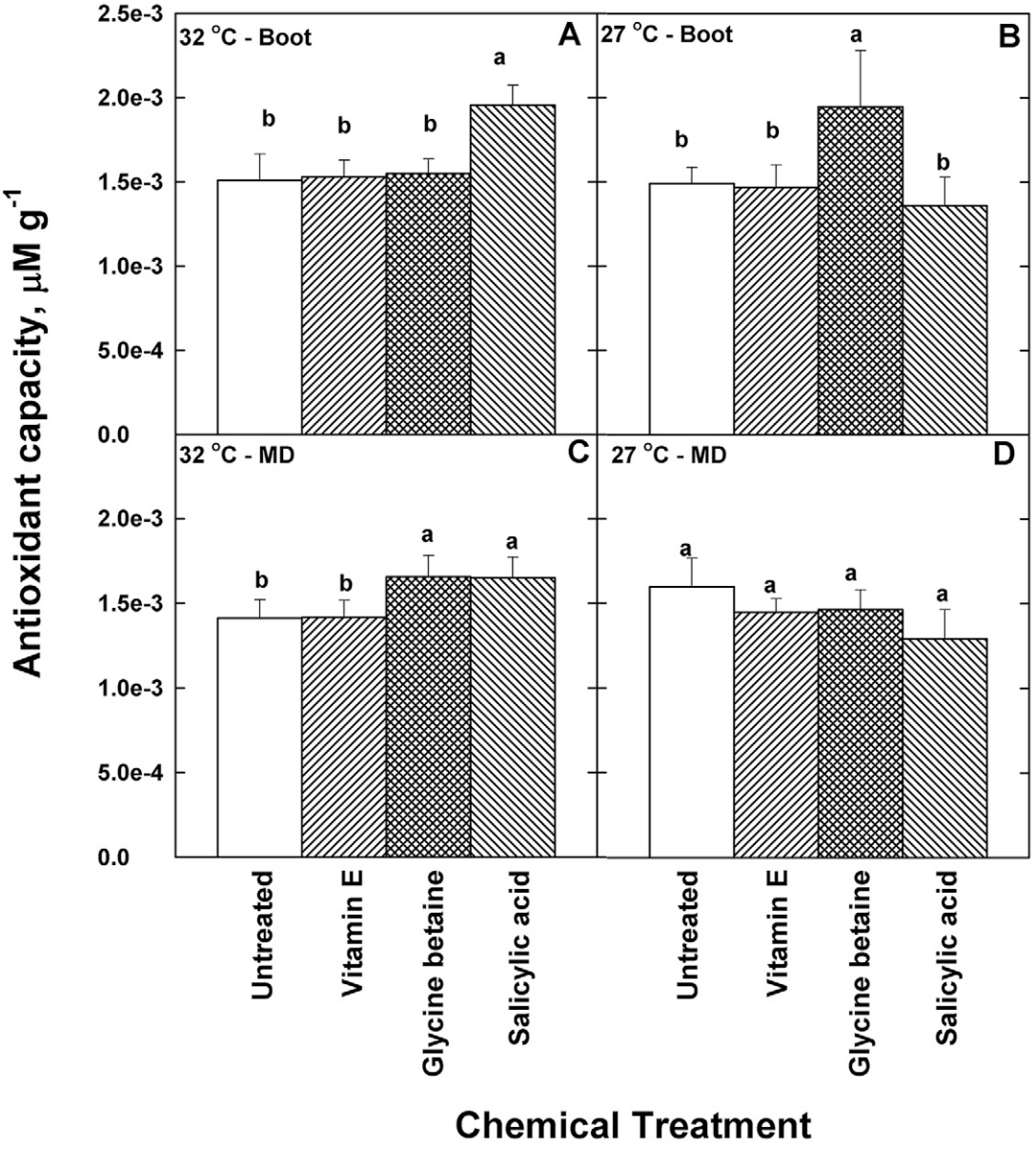

Figure 4. Effects of high nighttime temperature and exogenous effector (chemical) treatments on total antioxidant capacity of rice leaves. The total antioxidant capacity was measured at boot stage and mid-dough (MD) stage of the rice plants. There are four panels (A, B, C, D) in the graph, and the datum shown in each panel was analyzed separately. The error bars are shown if they are larger than the symbol, and represent SE. The values are averages of the results of three independent experiments. In total, there were thirteen replications for each treatment.

(Reynolds et al., 1994; Ismail and Hall, 1999), floral bud development (Ahmed and Hall, 1993), pollen viability (Prasad et al., 1999), flowering time and decreased seed size (Gibson and Mullen, 1996), and developmental period or rate in many crop species (Seddigh and Jolliff, 1984; Morita et al., 2005) in response to high temperature. In the present study, rice yield showed a negative association with leaf respiration rates and a positive association with leaf membrane stability. Previous studies reported a similar negative association between yield and respiration rates (Lambers, 1985) and positive association between yield and membrane stability (Reynolds et al., 1994; Ismail and Hall, 1999) in response to heat-stressed conditions. In addition, our results showed positive association between total antioxidant capacity and leaf membrane stability, suggesting membrane leakiness is associated with oxidative stress.

In the present study, plants treated with GB or SA showed increased total antioxidant capacities, thereby decreasing the extent of damage to the membranes caused by reactive oxygen species as a result of HNT, as indicated by MTS, hence minimizing yield losses under HNT. Previous studies have also shown an increase in endogenous antioxidant levels as a result of exogenous application of SA or GB (Chen et al., 1997; Diaz-Zorita et al., 2001). In the present study, both the exogenous effectors (GB and SA) were effective in slightly negating the negative effects of HNT on rice plants by reducing the respiration rates and electrolytic leakage. Increased antioxidant levels can detoxify superoxide radicals (Bowler et al., 1992), thereby preventing oxidative damage and protecting the membranes and enzymes (Diaz-Zorita et al., 2001), and hence decreasing the maintenance respiration, which is required for repair mechanisms of damaged membranes (Amthor and McCree, 1990). Based on our results, we propose that an increased antioxidant capacity as a result of application of GB or SA can reduce oxidative damage, thereby increasing MTS and reducing leaf dark respiration rates. Moreover, it has been stated that the antioxidant concentration of a plant is closely associated with its stress tolerance and survival (Smirnoff, 1995).

In conclusion, there were increases in leaf dark respiration rates and electrolytic leakage as a result of high

nighttime temperatures. Unlike other studies (Prasad et al., 2006), in which there was no relationship between electrolytic leakage and yield, the results of the present study indicated that electrolytic leakage increased with temperature. We were able to identify two potential exogenous effectors (GB and SA) to ameliorate the effects of HNT. Both compounds increased total antioxidant capacity of the rice plant, thereby presumably decreasing the leaf dark respiration rates and electrolytic leakage, hence increasing the yield.

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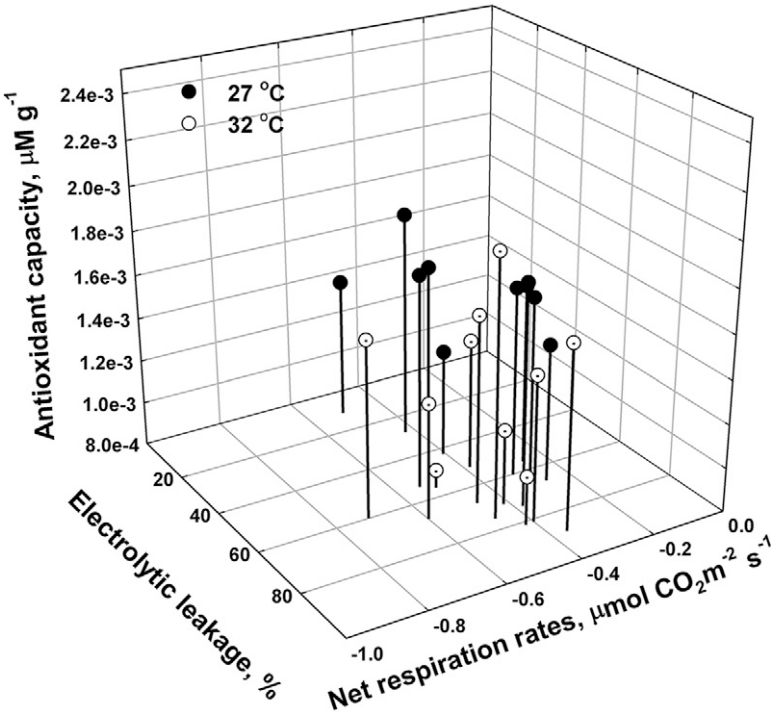

Figure 5. Leaf dark respiration rates, electrolytic leakage, and antioxidant capacity of leaf tissues of rice plants grown under ambient (27°C) or high (32°C) nighttime temperatures. Ten individual values for each parameter under each heat treatment are from untreated (no exogenous effector) plants, which were analyzed using Hotellings T-square analysis, a special case of multivariate analysis of variance (MANOVA). The plants' response to high nighttime temperature differed from the response to ambient nighttime temperature with respect to respiration rate, electrolyte leakage, and antioxidant capacity in combination.

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